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Piersma, T; Dekinga, A; van Gils, J A; Achterkamp, B; Visser, G H

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## Cost–benefit analysis of mollusc eating in a shorebird

### I. Foraging and processing costs estimated by the doubly labelled water method

Theunis Piersma<sup>1,2</sup>, Anne Dekinga<sup>1</sup>, Jan A. van Gils<sup>1,2,\*</sup>, Bart Achterkamp<sup>2</sup> and G. Henk Visser<sup>2,3</sup>

<sup>1</sup>Department of Marine Ecology and Evolution, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands, <sup>2</sup>Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands and <sup>3</sup>Centre for Isotope Research, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

\*Author for correspondence (e-mail: janvg@nioz.nl)

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#### Summary

Although the energy costs of foraging and food processing in vertebrates may be considerable, they have rarely been quantified separately. Here we present estimates for both cost factors based on a series of trials with a shorebird, the red knot *Calidris canutus*, fed natural and artificial prey types under naturalistic but fully controlled indoor aviary conditions. During eight 1-day trials we successfully manipulated the extent to which the five red knots were (1) actively probing and walking (i.e. foraging) and (2) actually ingesting prey (i.e. processing food) that was (3) either hard-shelled or not (i.e. crushing). Energy expenditures, estimated by the doubly labelled water (DLW) method, calibrated for use in this particular condition, varied between 1.5 and 4 W. A hierarchical analysis of variance indicated that the crushing of hard-shelled prey entailed no extra cost. We arrived at the following breakdown of cost components

under the thermoneutral conditions of the experiment: a cost of active rest/maintenance of 1.665 W, an additional cost of foraging of 0.602 W and an additional digestive processing cost of 1.082 W. These cost levels are all well within the range of expectation and are consistent with the results of a separate outdoor aviary experiment in which the thermostatic costs needed separate estimation. On the basis of the cost and performance functions of gizzards of different mass, it was shown that under the conditions of this experiment the red knots expended the bare minimum for a balanced budget, maintaining the smallest possible gizzard. Under field conditions a larger gizzard would be required.

Key words: aviary experiment, bivalve, *Calidris canutus*, cost–benefit analysis, digestion, doubly labelled water, energetics, food selection, foraging, prey quality.

#### Introduction

Cost–benefit analyses of patch and prey choice usually incorporate data on the energy value of various prey types on offer and on the time-cost of finding and ingesting such prey, but rarely include empirical estimates of the additional energy- and time-costs of the actual foraging behaviour and the internal processing of the prey (Stephens and Krebs, 1986; but see e.g. Rovero et al., 2000). Yet the time required for internal prey processing is an important variable in the cost–benefit equation (Kersten and Visser, 1996; van Gils et al., 2003b), especially in situations where foragers face digestive bottlenecks, which may be the case in the majority of foraging situations (Jeschke et al., 2002). In addition, in foragers eating prey that are refractory to digestion, the costs of maintaining an appropriate digestive tract may loom large in the energy budget (Lindström and Kvist, 1995; Karasov, 1996; Piersma et al., 1996; Starck, 1999). Obviously, different foraging styles may also have widely varying cost levels (Bryant and Westerterp, 1980; Masman et al., 1988; Godfrey and Bryant, 2000; Nolet et al., 2001).

A case in point is the shorebird species studied by our group during the last decade, the red knot *Calidris canutus*. This species is a specialised molluscivore (Zwarts and Blomert, 1992; Piersma et al., 1993a, 1998, in press), often eating rather poor quality prey types, i.e. low ratios of digestible flesh to shell (Zwarts and Wanink, 1991; Visser et al., 2000). Red knots have a large but variable digestive machinery, the gizzard and intestine especially showing strong variation (Piersma et al., 1993b; Dekinga et al., 2001; Piersma, 2002; Battley and Piersma, in press). Experimental work has indicated that digestive constraints, and possibly the costs of maintaining a large digestive tract, may be critical in shaping their foraging decisions (van Gils et al., 2003a). Here we present an analysis of energy expenditure of red knots in different foraging situations, estimated by the turnover of stable isotopes over experimental periods of 11 h. These energy expenditure levels are to be interpreted in the context of a cost–benefit analysis of the size and capacity of the digestive organ system.

## Materials and methods

### Experimental rationale

As is summarised in Table 1, in the course of this study we experimentally varied (1) the extent to which the birds needed to walk and probe in the soft sediment in order to obtain food (by enabling them to forage in a natural way in a tidal aviary or from a tray filled with prey), (2) the extent to which birds were eating and processing digesta, by presenting them or not with prey in the intertidal aviary or on the roost (in the latter case in the intertidal aviary they nevertheless actively walked and probed during the entire day), and (3) the type of food available: hard-shelled molluscs *versus* soft food pellets or dying open cockles (*Cerastoderma edule*) from which the meat was easily removed. In all cases, the birds were fed a given diet for at least 2 days, and the last day was always the day of measurement. The eight experimental treatments were presented to all birds in the same sequence over an experimental period of 40 days, with all but one cockle-diet trial taking place before the trials with food pellets. Although this schedule did not obey the experimental ideal of randomization, it had the benefit that experimental birds were better adjusted to diet type (cockles first, soft diets later; see Piersma et al., 1993b; Dekinga et al., 2001).

### Experimental arena: the NIOZ-indoor tidodrome

The trials were performed in either a large (7.3 m×8.0 m×3.0 m, length×width×height) indoor aviary or a smaller adjacent aviary (4.7 m×1.1 m×2.5 m, length×width×height). The bottom of the large aviary consisted of a layer (20 cm deep) of wet sand from the Wadden Sea. The basin could be filled with a layer of seawater, simulating high tides. The smaller, roost-site aviary, separated from the large tidal aviary with a sliding door, had a hard floor that was continuously wetted by seeping of seawater. Together, the two aviaries simulated an intertidal system with a low-tide foraging area and a high-tide roost, respectively; hence the name 'tidodrome'.

During the experiment the entire tidodrome was maintained at air temperatures between 16°C and 20°C, at the lower end of the thermoneutral zone of red knots (Wiersma and Piersma,

1994), and a relative humidity between 55% and 75%. Between 20.00 h and 08.00 h the large aviary was flooded with 14 cm seawater ('high tide'), during which the experimental birds used the small aviary with only small night-lights on. During the day the sandy bottom in the large aviary (the intertidal area) was exposed.

### Red knots and cockles

This study is based on measurements of five red knots *Calidris canutus* L. (three males, two females). A sixth bird (included in the analyses by Visser et al., 2000) had a breast wound. Although it participated in some of the trials, we have not used the data in the present analysis, as a bird with an incomplete plumage and a damaged skin might bias our estimates of the various cost factors.

The birds were captured in the Dutch Wadden Sea 9 months before the start of the experiment and were thus accustomed to captive conditions and frequent handling. Before the experiment the birds had been fed protein-rich food pellets *ad libitum* (Trouvit, Trout Nutrition, The Netherlands; containing 5.6% water, 48% crude protein and 12% crude fat). 3 weeks before the first trial, the birds were shifted to a diet of cockles only (*ad libitum*), to adjust their digestive tract to a diet of hard-shelled molluscs. Experiments have shown such adjustments to take less than a week (Dekinga et al., 2001). During this training period, the mean mass of the five birds decreased from 128.0±8.3 g (mean ± s.d.) to 113.4±8 g on the day before the first trial (5 January, 1998).

Prior to each of the separate trials, cockles were freshly collected from an intertidal flat close to the island of Texel in the western Dutch Wadden Sea. We determined the following relationship between shell length ( $L$ , mm) and the ash-free dry mass of the soft parts of the cockle ( $M_{AFD}$ , mg):  $M_{AFD}=0.006622 \times L^{2.840}$  ( $N=77$ ,  $r^2=0.89$ ); these values indicate that the collected cockles were in their normal winter condition (cf. Zwarts, 1991). The length of the cockles on offer varied between 6 mm and 15 mm, and they contained 68.6% water, 29.8% dry shell matter and only 1.6% ash-free dry matter of soft mass (i.e. digestible 'meat').

Table 1. Chronological summary of the characteristics of the aviary experiment with red knots

Experiment	Code	Day number	Activities expending energy		
			Foraging (probing and walking)	Digestive processing	Crushing shells and processing shell material
1	IF-C <sub>1</sub>	6	+	+	+
2	R-C	22	0	+	+
3	IF-C <sub>d</sub>	26	+	+	(0)
4	IF-C <sub>2</sub>	28	+	+	+
5	R-F	30	0	0	0
6	R-P	33	0	+	0
7	R-C <sub>m</sub>	35	0	+	0
8	IF-F	44	+	0	0

The code includes information on the experimental arena (IF, intertidal flat; R, roost), the presence and type of food (C, cockles; P, food pellets; F, fasting/no food) and the quality of the cockles on offer (subscript 1, poor quality; 2, medium quality; d, dying; m, meat).

*Experimental protocol and behavioural observations*

During trial 1 (IF-C<sub>1</sub>; Table 1) the birds fed on live cockles buried in the artificial sand flat. The birds had to walk and to probe to find the cockles. During trial 2 (R-C), trays with live cockles were offered on the roost, enabling the birds to eat the cockles without the additional effort of walking and probing. Trial 3 (IF-C<sub>d</sub>) consisted of feeding on the artificial mudflat on cockles that were dying because of anoxic conditions in the sediment. As they do in the field under similar conditions (A. Dekinga and A. Koolhaas, personal observation), the red knots used their time to remove the flesh from the open shells. Although they had to do the walking, there was little need for probing and they were not ingesting shells that needed crushing. Trial 4 (IF-C<sub>2</sub>) was a repeat of trial 1, except that the quality of the cockles on offer (meat per unit shell mass; see van Gils et al., 2003a) was 16% higher ( $P=0.005$ ). Trial 5 (R-F) studied the birds on the roost while fasting. Trial 6 (R-P) studied birds on the roost feeding on the soft pellets. Trial 7 (R-C<sub>m</sub>) consisted of red knots feeding on the roost on cockle meat removed from the shell after immersing them for a few seconds in boiling water. Trial 8 (IF-F) consisted of searching on the artificial mudflat for prey that were not there.

The eight trials were of equal length (11 h) and similarly structured, as follows. The evening before an experimental day, the birds were kept in the small aviary and fed cockles, supplemented with food pellets during the latter half of the study. In this way we made sure that the birds were able to balance their energy budget, as confirmed by the constant body masses maintained throughout this experiment (Visser et al., 2000). On the experimental day, birds were captured at 08.00 h and placed individually in small cardboard boxes, measuring 15 cm×15 cm×15 cm. In an order that was repeated at the end of the day, one by one the birds were taken out of the boxes, and when relevant a blood sample was taken to determine background levels of the isotopes (see below for procedure; 3 birds for each trial), and weighed to the nearest 0.1 g on a balance (model BD202; Mettler). In sequence, they were injected ventrally and subcutaneously with a precisely known amount of doubly labelled water  $^2\text{H}_2^{18}\text{O}_{18}$  (range: 0.4–0.9 g) using an insulin syringe weighed to the nearest 0.1 mg on a Mettler model AE160 balance before and after administration. The DLW-mixture was obtained by mixing  $^2\text{H}_2\text{O}$  (Aldrich, Milwaukee, USA) with  $\text{H}_2^{18}\text{O}$  (Rotem, Rehovot, Israel) to yield  $^2\text{H}$  and  $^{18}\text{O}$  concentrations of 30.4% and 62.7%, respectively, as assessed from isotope dilution measurements (Visser and Schekkerman, 1999).

To avoid potential problems of low isotope enrichment relative to background levels at the end of the trials, the highest doses were given in the trials with cockles (IF-C<sub>1</sub>, R-C, IF-C<sub>d</sub> and IF-C<sub>2</sub>). The dose was chosen such that the  $^2\text{H}$  and  $^{18}\text{O}$  enrichments of the final samples were at least 150 p.p.m. and 200 p.p.m. above the background levels, respectively. After 1 h in the dark cardboard box without food or water (usually at 9.10 h), the birds were reweighed and an (initial) blood sample was taken from the brachial vein after making a little puncture with a sterile needle (at least six 15 µl samples were taken into

glass capillaries that were immediately flame-sealed with a propane torch). Thereafter the birds were released in the experimental aviary until about 20.00 h, when they were recaptured by hand, and placed individually in the cardboard boxes. Repeating the morning sequence, the birds were reweighed and a (final) blood sample taken from the brachial vein in the other wing. Flame-sealed capillaries were stored at 4°C until the isotope analysis, which took place within 2 months of the experiment.

During each of the experimental days, behavioural observations were made through one-way screen windows in the experimental aviaries. During the 11 h experimental periods, a scan was recorded every 5 min during which the behaviour of each individual was categorized as either foraging (i.e. walking and active probing or ingesting), resting (subdivided into standing, sleeping and preening) or – rarely – flying. Assuming that scored activities are representative of the previous 5 min interval, time budgets were calculated for each bird (and for each trial). Individual birds were closely observed four times per hour for 1 min to determine the number of prey swallowed, which enabled us to estimate daily intake rate.

*DLW analyses and calculations of daily energy expenditure*

Samples were analysed in quadruplicate at the Centre for Isotope Research (CIO) at the University of Groningen, following the procedures described in detail by Visser and Schekkerman (1999), Visser et al. (2000) and Jenni-Eiermann et al. (2002). Briefly, as a first step, blood samples were cryogenically distilled in a vacuum line. Next, the  $^{18}\text{O}/^{16}\text{O}$  isotope ratio was determined in  $\text{CO}_2$  gas (using the  $\text{CO}_2$  equilibration method) and, after reduction of the water sample over a hot (800°C) uranium oven, the  $^2\text{H}/^1\text{H}$  isotope ratio was determined in  $\text{H}_2$  gas using a SIRA 10 Isotope Ratio Mass Spectrometer (Manchester, UK). In each batch, a diluted sample of the doubly labelled water (DLW) injectate, together with four internal laboratory standards that covered the observed enrichment range of the blood samples, were analysed. These standards were calibrated against IAEA (International Atomic Energy Agency) standards.

The amount of body water in each individual was determined using the principle of isotope dilution. For a detailed presentation of the calculated sizes of the birds' body water pools, see Visser et al. (2000). Fractional isotope turnover rates for each isotope were calculated based on the isotope enrichment of the initial and final samples, the population-specific average enrichment of the background samples, and the time interval elapsed between the taking of the initial and final blood samples (equation 2, Visser et al., 2000). The coefficients of variation of the final  $^2\text{H}$  and  $^{18}\text{O}$  measurements above the background values were 0.8% and 1.3%, respectively. The rates of  $\text{CO}_2$  production were calculated for each measurement period using Speakman's equation 7.17 (Speakman, 1997), which takes into account fractionation effects of the  $^2\text{H}$  and  $^{18}\text{O}$  isotopes, assuming that 25% of the water efflux was lost through evaporative pathways. In an accompanying study, during which the DLW

method was validated against respiration gas analyses in birds fed different diets (to induce different rates of water efflux), this equation has proved to yield the most robust estimates of CO<sub>2</sub> production rates (G. H. Visser, A. Dekinga, J. A. Gessaman, E. R. Th. Kerstel, and T. Piersma, manuscript in preparation), and measurements were taken over the same time intervals as applied in the present study.

As a last step, the rates of CO<sub>2</sub> production were converted to energy expenditure values using an energy equivalent of 27.3 kJ l<sup>-1</sup> CO<sub>2</sub>, which is appropriate for diets with very high protein content (Gessaman and Nagy, 1988).

### Statistics

Because of the hierarchically structured design of the experiment (individual observations nested within trials), we used a hierarchical linear model (i.e. mixed model or multilevel model) to test for the effects of the treatments on metabolic rate. These treatments (foraging, crushing and digestive processing) were entered as categorical variables (either 0 or 1), and trial, denoting the nesting of the observations, was entered as the identifier variable. We allowed the intercept to vary across trials, i.e. we used a random intercept model. The test was performed using the MIX procedure in SYSTAT 10 (SPSS Inc., Chicago, IL, USA). We used the same type of model to test for the success of the manipulations. The percentage of time spent foraging was arcsine-square-root transformed before analysis.

### Results

Each of the manipulations with respect to activity and intake (Table 1) was apparently successful (Fig. 1). The percentage of

time spent foraging was higher when the birds had the indoor intertidal sand flat available (foraging=yes) than when they were restricted to the small roosting aviary (foraging=no;  $P=0.001$ ). Unsurprisingly, they did obtain higher ash-free dry mass intake rates when they were offered prey (digestive processing=yes) than when they were not (digestive processing=no;  $P=0.006$ ). And, the red knots indeed achieved a higher shell mass intake rate when intentionally offered shelled prey (crushing=yes) than when they were not (crushing=no;  $P<0.001$ ).

Under this range of experimental conditions the energy expenditures of red knots varied between 1.5 W when not foraging, to over 4 W when working and eating (Fig. 2). The energy expenditures in different foraging contexts (Fig. 2) also indicate (1) that foraging activity (even when not accompanied by actual food intake) is more costly than active rest, (2) that the act of ingesting food items, whether these items are of a soft or a hard-shelled nature, adds importantly to the expenditure level, but (3) that the additional costs of crushing the hard-shelled prey items are small or negligible. A hierarchical analysis of variance (ANOVA; Table 2A) confirms that the additional effect of crushing on energy expenditure is non-significant, which leads to a simplified model that only considers whether food is eaten regardless of prey type (Table 2B).

To see whether the cost of crushing was nevertheless significant in more controlled pairwise comparisons, we tested for differences in metabolic rates between trials that *only* differed in whether prey needed to be crushed or not (again using a hierarchical linear model). For both comparisons there again appeared to be no significant metabolic cost of crushing ( $P=0.887$  for the comparison between trials 1 and 4 vs 3;  $P=0.670$  for trial 2 vs 6 and 7).

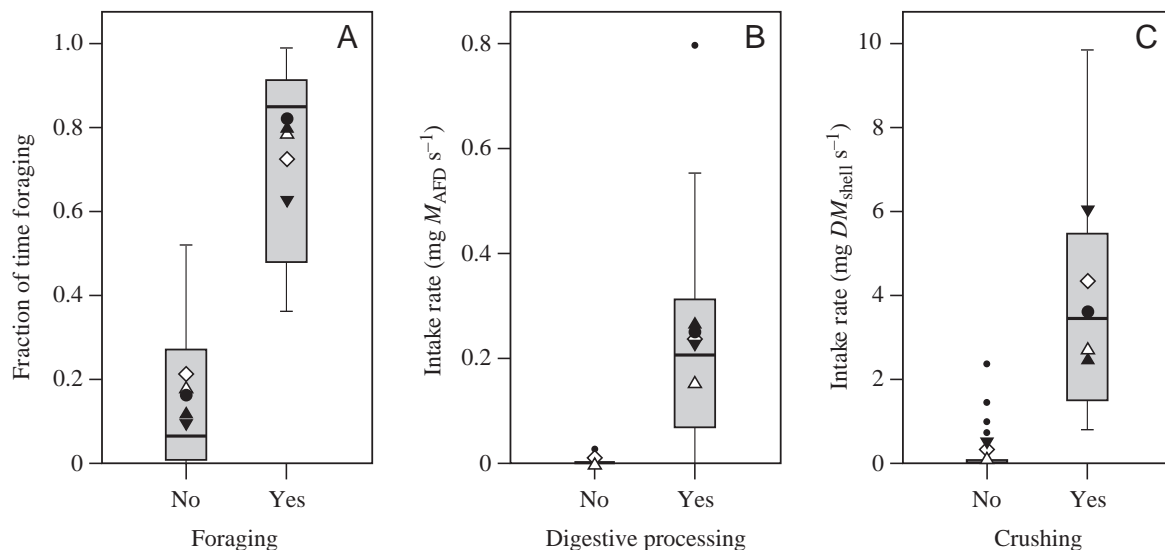


Fig. 1. Methodological graph showing the degree of success of the three different treatment categories: (A) fraction of time spent foraging, (B) measurement of intake rate (ash-free dry mass  $M_{AFD}$  per second), as an indication of the extent of energy intake, and (C) measurement of intake rate (shell dry mass  $DM_{shell}$  per second) to indicate shell crushing. These box-and-whisker plots give median (horizontal line within box), interquartile range (box), range (bars), and outliers (small filled circles) among all relevant trials. Larger symbols indicate mean values per individual per treatment category.



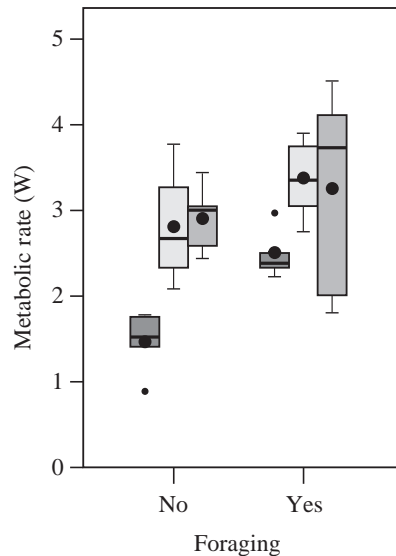


Fig. 2. Rates of energy expenditure (W) measured with the doubly labelled water method as a function of whether the birds were foraging (horizontal axis), subdivided into trials when they were not crushing or processing digesta (dark grey boxes), processing but not crushing (open boxes), or processing and crushing (light grey boxes). Large filled circles are least-square means and small filled circles are outliers (see Fig. 1 for a further description of box-and-whisker plots).

The cost of maintenance plus limited activity is estimated at 1.665 W (Table 2B). Foraging adds 0.602 W and digestive processing another 1.082 W. Accounting for these three factors, only a small part of the overall variance in energy expenditure remained unexplained. Note that the standard errors were more or less similar for the three components, but were largest relatively for foraging, the smallest component of the energy budget.

Table 2. Analysis of variance in metabolic rate (W) estimated by a hierarchical linear model, where individual observations are nested within experimental treatments

Variable	Estimate	Standard error	Z-score	P-value
A Intercept	1.659	0.323	5.140	<0.001
Foraging	0.613	0.299	2.050	0.040
Digestive processing	1.105	0.373	2.963	0.003
Crushing	-0.045	0.345	-0.131	0.895
Residual variance	0.319	0.080	4.000	<0.001
B Intercept	1.665	0.320	5.198	<0.001
Foraging	0.602	0.286	2.100	0.036
Digestive processing	1.082	0.331	3.270	0.001
Residual variance	0.319	0.080	4.000	<0.001

The variable 'crushing' explained no significant variance in model A and is therefore omitted from the analysis presented in model B.

## Discussion

### Cost estimates for foraging and food processing

Under the thermoneutral conditions of this aviary experiment the incremental cost of foraging behaviour (walking and probing) is the lowest among the three cost factors (Table 2B). This is perhaps to be expected in a bird species designed for low-cost terrestrial locomotion (Bruinzeel et al., 1999). Using the overall mean body mass of the experimental birds (122.2 g) and a measured walking speed ( $0.072 \text{ m s}^{-1}$ ), the intraspecific allometric relationship of Bruinzeel et al. (1999) predicts a cost of walking of 0.136 W. Given a net foraging cost of 0.602 W, the cost of probing can thus be estimated at  $0.602 - 0.136 = 0.466 \text{ W}$ . This means that the summed costs of making rapid probing movements with bill and head (ca. 10 Hz; see Piersma et al., 1995) to overcome the resistance of the soft sediment when inserting the bill tip (Piersma et al., 1998), as well as retrieving the buried prey, are 3–4 times as high as those of walking.

The digestive processing cost is mainly represented by the energy (heat) losses due to inefficiencies in intermediary metabolism (Klasing, 1998). This cost, often called 'Heat Increment' or 'Heat Increment of Feeding' (HIF), was estimated at 17% of the metabolizable energy intake in kestrels *Falco tinnunculus* (Masman et al., 1989) and at 20% in brent geese *Branta bernicla* (Sedinger et al., 1992). These values are lower than the contribution of the processing cost to energy expenditure estimated here (32%). This high value may be due to the highly proteinaceous diet offered to red knots. In chickens, HIF is considered to be 30% for protein, 15% for starch and 10% for lipids (Klasing, 1998). Alternatively, since the habit of ingesting entire shell fish is accompanied by the ingestion of huge amounts of water (up to 300 g per trial; Visser et al., 2000), heat loss to the ingested cold water pool (up to 0.58 W) could be part of the explanation as to why the digestive processing cost is relatively high in red knots.

Crushing hard-shelled prey did not add significantly to the hierarchical linear model of cost factors to explain the variance in energy expenditure levels (Table 2A). Apparently, the activity of the muscular gizzard is small compared with the other costs of digestion that relate to processes in the intestinal tract and the liver. Considering the limited action taking place in the gizzard of red knots (a few seconds of crushing immediately followed by the evacuation of prey remains into the intestine, rather than the longer lasting pre-digestion in the proventriculus and grinding in the gizzard of many other birds; Klasing, 1998), the small and immeasurable cost of 'crushing' should perhaps not have surprised us. This low *energetic* cost of crushing is in sharp contrast to the high overall *time* costs involved in the digestive processing of shelled prey items (van Gils et al., 2003a).

Finally, we note that the cost of active rest (i.e. 1.665 W, the intercept of the hierarchical linear model), is much higher than the predicted value for basal metabolic rate (BMR) of ca. 1 W (Piersma et al., 1996). Although the birds were maintained at thermoneutrality, they did not sleep for most of the time (as

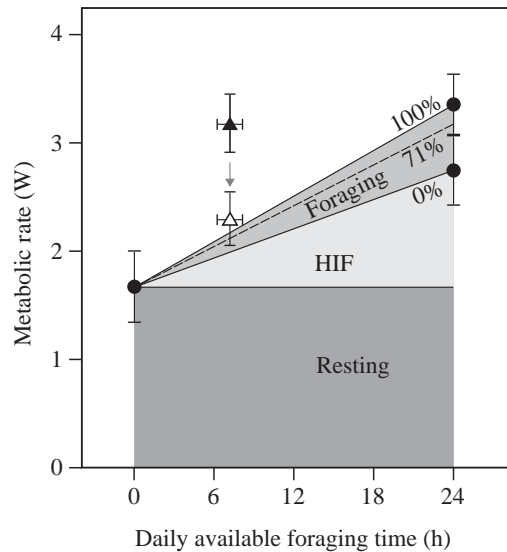


Fig. 3. A comparison of the estimated metabolic rates found in this indoor study (filled circles) with a doubly labelled water-estimate obtained in a similar study on an outdoor intertidal flat (Poot and Piersma, 1994; filled triangle). In this outdoor study it was calculated that the thermoregulatory costs amounted on average to 0.88 W. Thermoregulatory costs were absent in our indoor study. We therefore subtracted 0.88 W from the outdoor metabolic rate (open triangle) to make the metabolic rates from both studies comparable (note that this assumes the additive energy budget model is operative – see main text). This ‘thermoneutral’ outdoor metabolic rate is correctly predicted by combining the three indoor activity-specific cost estimates with the outdoor time budget (7.2 h of available foraging time per day, of which 71% was actually spent foraging). Values are means  $\pm$  S.E.M. HIF, heat increment of feeding.

they would during the measurement of BMR in darkness), and although they did not walk much, they went through daily routines such as bathing and preening. Just being awake and carrying out minimal activities apparently costs about half as much as being asleep (BMR).

#### Validation in an outdoor experiment

The present study took place in thermoneutrality, a condition that red knots only encounter in the tropics (Wiersma and Piersma, 1994). In colder environments, knots need to produce heat to stay warm. Whether in our total budget calculations we can simply add these thermoregulatory costs to the estimated foraging and processing costs depends on the type of energy budget model that is used. Additive models assume that this thermoregulatory heat needs to be generated as an extra by shivering; substitution models assume that heat generated as a byproduct of metabolic processes can (partly) substitute for the thermoregulatory heat. A preliminary study under outdoor conditions using similar experimental techniques and isotope analyses (Poot and Piersma, 1994; Fig. 3) allowed us a preliminary test among the two models.

Red knots were allowed to feed on shellfish buried in an intertidal flat (comparable to our IF-C conditions in Table 1).

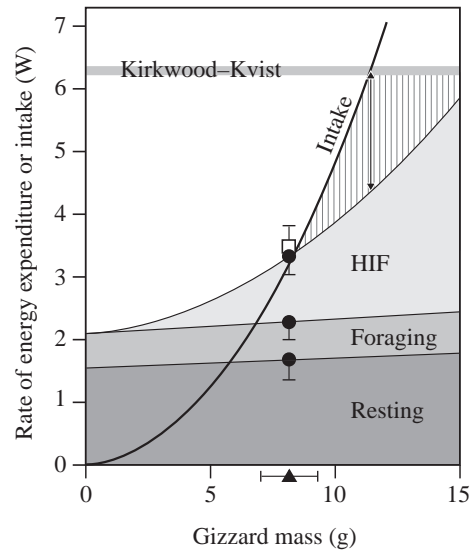


Fig. 4. Energy budget for knots feeding on poor-quality cockles as a function of gizzard size. For the experimental birds, gizzard mass =  $8.13 \pm 0.98$  g (mean  $\pm$  S.E.M.), we plotted the cumulative cost levels for the three components that we measured (rest, foraging and heat increment of feeding, HIF; filled circles; figures as in Table 2B). Values are means  $\pm$  S.E.M. Theoretically, each of these cost levels would increase with gizzard mass (with concomitant increases in intestine mass, Table 3), since larger gizzards require larger maintenance costs (Piersma et al., 1996), larger gizzards increase the cost of walking (Bruinzeel et al., 1999), and larger gizzards quadratically increase HIF (since intake increases quadratically with gizzard size; see van Gils et al., 2003a). Two digestive constraints are presented: (1) the gizzard-size independent rate at which flesh can be digested (horizontal grey bar; based on Kirkwood, 1983 and Kvist and Lindström, in press), and (2) the rate at which shell material is processed, a quadratic function of gizzard size (solid curve; van Gils et al., 2003a). Intake rates measured in the two most ‘natural’ trials (means of trials IF-C<sub>1</sub> and IF-C<sub>2</sub>; open square) appear to be set by this ‘gizzard-size constraint’. The hatched area below the two digestive constraints but above the cumulative cost levels gives the scope for a positive energy budget and is maximal at the arrow.

Over the 24 h period of the DLW measurements, these birds only had access to the flat for 7.2 h on average, when they were actively feeding for 71% of their time. Assuming that these birds were maximizing their daily net energy intake, the remaining 29% of their available foraging time was probably lost to digestive breaks (cf. van Gils et al., 2003b). Therefore, foraging costs (0.602 W) were made for only  $71\% \times 7.2$  h per day, HIF costs (1.082 W) for 7.2 h per day, and resting costs (1.665 W) for 24 h per day. Adding a thermoregulatory cost of 0.88 W that Poot and Piersma (1994) estimated from measurements with heated taxidermic mounts (see Wiersma and Piersma, 1994), the additive energy budget model predicts a daily average metabolic rate (ADM) of 3.00 W (Fig. 3). The substitution model predicts an ADM of 2.72 W, which is based on the assumption that a 100% of the heat increment of feeding and 30% of the heat generated by walking is substitutable (the latter estimate is based on calculations by

Bruinzeel and Piersma, 1998). The outdoor data averaged  $3.17 \pm 0.27$  W (mean  $\pm$  S.E.M.,  $N=12$ ), a value that is not different from the predictions of either the additive model ( $P>0.45$ ) or the substitution model ( $P>0.10$ ). Although this result does not allow us to conclusively state whether thermoregulatory heat can be substituted for, the data indicate that the different cost components estimated indoors may be robust. This should allow us to use them as predictions for field situations (van Gils et al., 2003a).

#### A cost-benefit analysis

With the estimates for the different cost components at hand (Table 2B), in combination with the empirical function relating rate of energy intake to gizzard mass (van Gils et al., 2003a), we could calculate an energy-based cost-benefit analysis of the gizzard mass of red knots (Fig. 4); for the details of this analysis, see Appendix in the accompanying paper (van Gils et al., 2003a). With an increase in gizzard mass, two processes are implicated. Foremost, intake rate can be increased (being a quadratic function of gizzard mass; van Gils et al., 2003a). Secondly, a portion of this extra gain disappears due to increases in HIF, resting and foraging costs. HIF increases simply because food is processed at a higher rate, resting costs increase (slightly) because larger organs require larger maintenance costs (BMR), and foraging costs increase (slightly) because higher locomotory costs are required to carry around the heavier body. These latter two mass-related costs increase at double speed since a change in gizzard mass is accompanied by a similar change in intestine mass (Table 3; Battley and Piersma, in press). Under the conditions of the experiment, for gizzard mass higher than 8 g, the potential intake rate actually exceeds the concomitant cost levels until a plateau (set by other parts of the digestive system) is reached at gizzard mass 11–12 g. At this level net intake rate would be maximised (van Gils et al., 2003a). Fig. 4 shows that the experimental knots kept their energy budget just balanced during the foraging period. It follows that the birds would have been unable to survive on this prey type in the field, as the energy budget while foraging should be positive to compensate for the loss of energy during the high-tide roosting period. We predict that red knots facing bivalve prey of similar quality in the field as in the experiment would have larger gizzards. This appears to be the case (van Gils et al., 2003a).

Table 3. Results of a general linear model (GLM) of log-transformed intestine mass (g) as a function of log-transformed gizzard mass (g) in red knots

	d.f.	SS
Gizzard mass	1	203.179
Error	262	5.637
$r^2$		0.973

d.f., degrees of freedom; SS, sum of squares.

$N=263$ ;  $P<0.0005$ ; slope= $1.034 \pm 0.011$  ( $\pm$ S.E.M.); intercept set at zero).

Data are from Battley and Piersma (2004).

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